(19) Europäisches Patentamt
Europ an Pat nt Office
Offic européen des brevets

(11) Publication number: **0 277 688 B1**

(12) **EUROPEAN PATENT SPECIFICATION**

(45) Date of publication of patent specification: **27.10.93**

(51) Int. Cl.5: **C12P 11/00**, C12P 33/00, A23L 1/226, C07C 45/78

(21) Application number: **88200141.5**

(22) Date of filing: **27.01.88**

The file contains technical information submitted after the application was filed and not included in this specification

(54) Method for preparing thiol compounds.

(30) Priority: **30.01.87 NL 8700240**

(43) Date of publication of application:
10.08.88 Bulletin 88/32

(45) Publication of the grant of the patent:
27.10.93 Bulletin 93/43

(84) Designated Contracting States:
AT BE CH DE ES FR GB GR IT LI LU NL SE

(56) References cited:

J. of Biol. Chemistry, vol. 253, no. 24, 25th Dec. 1978, pp. 8854-8859, US; M. Tateishi et al.

Microecology and Therapy, vol. 15, 1985, pp. 261-266, Institut für Mikroökologie, Herborn-Dill, DE; G.L. Larsen et al.

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EP 0 277 688 B1

Descripti n

The invention relates to a method for preparing thiol compounds.

In Pesticide Biochemistry and Physiology 14, pp. 50-61 (1980) the in vitro metabolism of pentachloronitrobenzene (PCNB) into pentachloromethylthiobenzene (PCTA) by means of an enzyme system obtained from onions is described. More particularly, this reference relates to the in vitro preparation of PCTA from PCNB at a pH of 7.9 by means of an enzyme system which contains dithiothreitol, glutathione and S-adenosylmethionine. Said enzyme system was prepared from onion roots by ammonium sulphate fractionation and differential centrifugation. The enzyme system contained glutathione-S transferase activity with PCNB, C-S-lyase activity (also termed β-lyase activity) with S-(pentachlorophenyl)cysteine, S-adenosylmethionine-methyl transferase activity with pentachlorothiophenol (PCTP) and probably a few other peptidase activities. The yield of the thiol compound concerned, namely pentachlorothiophenol (PCTP) is, however, negligible in this known method compared with the yield of PCTA (see page 55, right-hand column, lines 10-13 from bottom) so that this method is considered unsuitable for preparing thiol compounds.

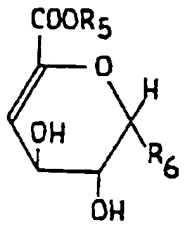
In Journal of Biological Chemistry, vol.253, 24, pp.8854-8859 (1978), the cysteine conjugate β-lyase in rat liver is described. This enzyme catalysing cleavage of the thioether linkage in cysteine conjugates has been purified about 500-fold from rat liver cytosol. However, according to the chapter "Assay Methods" (page 8855) the obtained thiol compounds were directly methylated whereafter the methylated derivatives were identified by spectroscopy methods.

Microecology and Therapy, vol. 15, 1985, pp. 261-266 discloses a pathway for the introduction of sulfur in the form of thiol-, methylthio-, methylsulfinyl- or methylsulfonyl-groups into xenobiotics. The β-lyase in the gastrointestinal bacteria is a key enzyme in this biotransformation as it converts S-cysteine conjugates into thiol containing metabilites which will usually - on account of the toxic properties of thiols - undergo further metabolism.

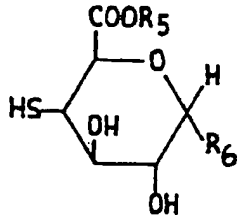
A method defined in the introduction has now been found which is characterized in that cysteine is coupled by an addition reaction of cysteine to a compound having the formula $(R_1)(R_2)C=C(R_3)\text{-}CO\text{-}R_4$ in which the symbols R_1-R_4 represent a hydrogen atom or an optionally saturated and/or heterogeneous hydrocarbon group or, together with the carbon atom to which the symbols are bonded, form one or two, optionally saturated and/or heterogeneous ring systems and subsequently the cysteine conjugate obtained is reacted with a β-lyase to form the relevant thiol compound.

For example, in the compound having the formula $(R_1)(R_2)C=C(R_3)\text{-}CO\text{-}R_4$ the symbols R_1-R_4 represent a hydrogen atom, an alkyl group containing 1-5 carbon atoms, an alkenylene group containing 2-5 carbon atoms, a cycloalkyl or cycloalkenyl group containing 5-10 carbon atoms or an aryl group containing 6-10 carbon atoms, which abovementioned groups may be substituted by halogen atoms and/or one or more groups containing carbon, nitrogen, sulphur, oxygen and/or halogen atoms. Preferably, the symbols R_2 and R_3 represent a hydrogen atom or an alkyl group containing 1-3 carbon atoms and R_4 an optionally heterogeneous hydrocarbon group bonded via an -O- bridge.

For example, unsaturated sugars having the formula



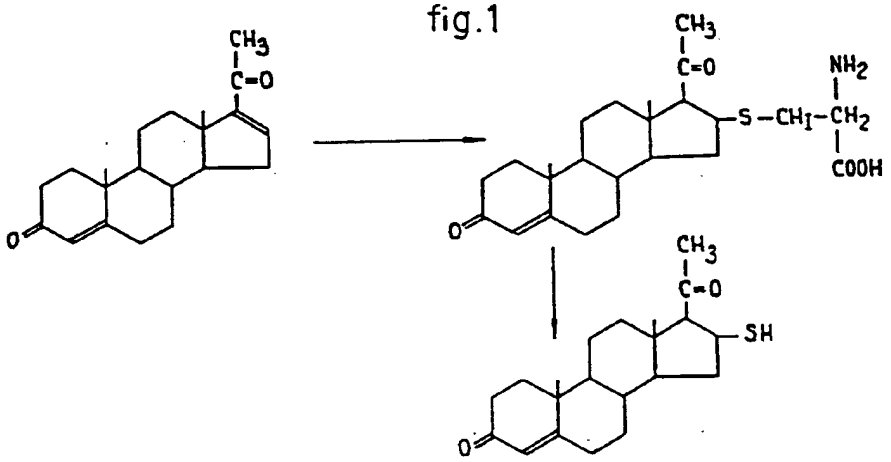
in which the symbol R_5 represents a hydrogen atom, an alkyl group containing 1-24 carbon atoms or an alkaline ion and R_6 represents a group consisting of 1-7 monosaccharides selected from the group consisting of glucose, mannose, galactose, arabinose, fucose, xylose, rhamnose, uronic acids and derivatives thereof like the acetates, pyruvates, amines and sulphates are also suitable as starting material for the addition reaction of cysteine. Preferably R_6 represents a glucose-rhamnose-glucose group. The obtained cysteine-conjugates are simply convertable to compounds with the formula



having flavouring properties.

Many types of cysteine conjugates are known as such from the prior art. For example, the preparation of such a cysteine conjugate is known from Applied and Environmental Microbiology, May 1985. pp. 1146-1153. In this reference, 16-dehydroprogesterone, in particular, is converted with L-cysteine in a non-enzymatical manner into 16-S-cysteinyl-progesterone. Said cysteinyl compound can be converted in the presence of β-lyase into 16-mercaptoprogesterone by means of the second stage of the method according to the invention. The diagram below illustrates the synthesis route described above:

fig.1



The thiol steroid shown above has specific pharmacological properties.

The formation of cysteine conjugates of 3-(3,4-dihydroxyphenyl)analine is reported in Biochimica et Biophysica Acta 672 (1981), pp. 151-157. As indicated on page 155 of this reference, polyconjugates can also be obtained in addition to some monoconjugates. These singly or multiply conjugated compounds can also be converted by means of the β-lyase to be used according to the invention into the corresponding mono- or polythiol derivatives.

Reference may be made to the following additional references relating to specific cysteine conjugates or derivatives derived therefrom:

1) J.Chem.Soc.Chem.Commun. 1986, pp. 1331-1333;
2) Journal of Food Science, vol. 51, no. 5, 1986, pp. 1191-1194;
3) Planta (1986) 169: 208-215; and
4) Carbohydrate Research 142 (1985), pp. 93-105.

The cysteine used in the method according to the invention has the formula $HS-CH_2-CH(NH_2)-COOH$. In view of the spectrum of activity of the β-lyase to be used in the method according to the invention, L-cysteine is used.

The β-lyase (also termed C-S-lyase or cysteine conjugate β-lyase) to be used in the method according to the invention is an enzyme dependent on a pyridoxal 5-phosphate (vitamin B6). In addition to being present in a large number of intestinal bacteria (in 24 out of the 43 intestinal bacteria investigated), the β-lyase is also present in some vegetable and animal cells (Larsen G.L., "Distribution of cysteine conjugate B-lyase in gastro-intestinal bacteria and the environment, Xenobiotica 15, 199-209 (1985)). The bacterial β-lyases are able to convert a wide spectrum of substrates, in particular both S-alkyl- and S-arylcysteine conjugates, whereas the spectrum of activity of β-lyases of vegetable or animal origin is limited. Measured

with the cysteinepropachlor conjugate (an S-alkylcysteine conjugate), the β-lyase originating from the anaerobic intestinal bacterium Eubacterium limosum is the most active enzyme and has the lowest substrate specificity (Larsen, loc. cit.). If, however, the conversion of S-(2-benzothiazolyl)cysteine (an S-arylcysteine conjugate) is examined, it emerges that the β-lyase from an anaerobic Fusobacterium species has virtually an identical activity. β-lyase from F.necrophorum and E.limosum differ not only in substrate specificity, but also in size, namely 228 kd and 75 kd (2x38 kd) and also in stability. The enzyme from F.necrophorum requires pyridoxal 5-phosphate for stability but is then also more stable to heat. β-lyases from E.limosum and F.varium exhibit no activity with D-cysteine conjugates and have, in general, a lower activity for S-alkylcysteine conjugates than for S-arylcysteine conjugates.

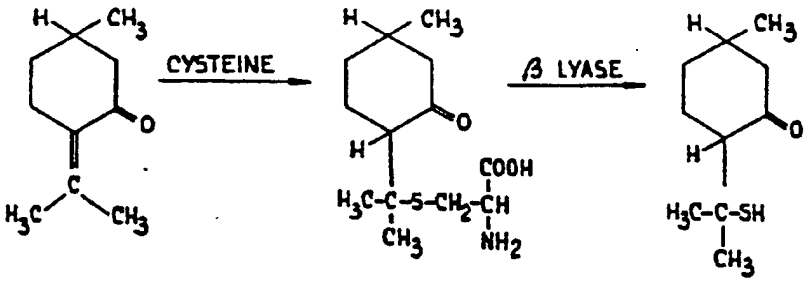
The isolation of β-lyase from both E.limosum and F.varium does not have to be carried out under anaerobic conditions. This indicates that the enzyme is not sensitive to oxygen. It also emerges from the isolation method that the enzyme is located in the cell. The second step described above of the method according to the invention can therefore be carried out with purified/extracted β-lyase or, if the substrates are absorbed by the bacterial cells and are converted therein, with the respective bacteria themselves.

The method according to the invention results in many types of thiol compounds with divergent applications. Examples of substances to be prepared pertain to the field of perfumes and flavourings (p-mentha-8-thiol-3-one, damascone derivative), pharmacological steroid compounds and repellants (Warburganal).

The invention is explained on the basis of the examples below.

Example I

In this example, the starting point is pulegone, which is converted via S-cysteinyl-pulegone into p-mentha-8-thiol-3-one. This preparation is illustrated in the diagram below.



Stage 1) Preparation of S-cysteinyl-pulegone.

12.2 g of L-cysteine (0.1 mol) (high purity analytical grade supplied by Fluka A.G.), 16.3 ml of pulegone (0.1 mol) and 2.0 g of $KHCO_3$ (0.02 mol) were stirred for 22 hours in 100 ml of H_2O at room temperature. The yoghurt-like mixture, which was no longer stirrable; was then allowed to stand for 3 days. The product obtained was then filtered off by suction and washed respectively with 100 and 2 x 50 ml of H_2O. After drying over $CaCl_2$ in vacuo, the product was washed with acetone. The yield was 17.9 g. Appendix 1 shows the 90 MHz H-NMR spectrum of the product obtained.

More particularly, an elementary analysis of the product purified by thin-layer chromatography clearly indicates a 1:1 reaction product.

Elementary analysis (carried out in duplicate).	
Found:	Calculated (substance + 1/2 mol of H_2O):
%C: 54.76	55.29
%H: 8.36	8.57
%N: 5.01	4.96
%O: 19.60	19.83
%S: 11.13	11.35

Stage 2) Splitting of the S-cysteinyl-pulegone.

The organism used in this stage was Eubacterium limosum having the ATCC no. 10825. Said organism was cultured under anaerobic conditions at 37 °C on a P-medium which had the composition below:

Composition of P-medium:	
Casein peptone	(Difco) 10 g/l
Beef extract	(Difco) 3 g/l
Yeast extract	(Difco) 3 g/l
Glucose	(Merck) 2 g/l
Tween 80	(Serva) 1 g/l
Cysteine-HCl	(Fluka) 0.5 g/l
Resazurin	(Serva) 0.25 g/l
Salt solution	(analytical grade) 40 ml/l
Final pH: 7.2	

The salt solution consisted of:	
$CaCl_2$	0.2 g/l
$MgSO_4.7H_2O$	0.2 g/l
K_2HPO_4	1.0 g/l
KH_2PO_4	1.0 g/l
$NaHCO_3$	10.0 g/l
NaCl	2.0 g/l

The cell material for producing β-lyase was obtained by culturing E.limosum (3% inoculation) on the abovementioned P-medium in serum bottles having a capacity of 300 ml. By filling the bottle with P-medium to a few centimetres below the rim, the medium became sufficiently low in oxygen as a result of sterilization to make growth of E.limosum possible. After an incubation time of 1 day at 37 °C, the cells were harvested by centrifuging them at 50,000 x g for 20 minutes. The cells were subsequently washed twice with a buffer having a pH of 7 which contained 50 mM of phosphate and 50 mM of pyridoxal-HCl. The pellet (approx. 1 g wet weight from 300 ml) was taken up in 10 ml of buffer.

S-cysteinyl-pulegone (0.3 g/l = 1.1 mM) was converted in the buffer with the concentrated cell Suspension of E.limosum described above (final concentration: 1.6 mg dry weight/ml). The reaction was carried out for 1 hour at 30 °C and was terminated by centrifuging the reaction mixture for 5 minutes at 11,000 x g.

As a control, two tests were carried out:

a) As a control, boiled cells (denatured enzymes) were used in the test described above.

b) In order to be able to assess whether the SH product (p-mentha8-thiol-3-one) had been converted by the S-methyl transferase into the S-methyl product (p-mentha-8-thiomethyl-3-one), the cells were also incubated with p-mentha-8-thio-3-one.

The results of gas chromatography analysis of this example (sample no. 1) and the two control tests (samples 2 and 3) are shown in Appendix 2.

To carry out the abovementioned gas chromatographic analysis, 1 part of chloroform ($CHCl_3$) was mixed with 1 part of the reaction mixture obtained. 1 µl of this extract was injected into a gas chromatograph having a 20 M carbowax column, (1.3 m RVS, column temperature: 145 °C, injection port and TCD temperature: 160 °C).

Example II

The method according to Example I was repeated, but with the difference that, instead of being carried out on a 1 ml scale, the test was carried out on a 10 ml scale. In this test, the cells were used in a double concentration, viz. 3.2 mg dry weight/ml and the incubation was carried out for 3 hours at 37 °C. For a gas chromatographic analysis, a sample (sample B) was taken from this in the following manner.

One part of dichloromethane (CH_2Cl_2) was mixed with 4 parts of the reaction mixture. 0.4 µl of this extract was injected into a Varian gas chromatograph in which a 10 % FFAP-chromosorb was provided in a WAW column (2m RVS, i.d. 1/8") (column temperature: 160 °C; injection port and FID temperature: 180 °C).

As a comparison, in addition to the gas chromatogram of sample B shown in Appendix 3 as a control, the gas chromatograms of a) p-mentha-8-thiol-3-one, b) p-mentha-8-thiomethyl-3-one, c) pulegone, and d) S-cysteinyl-pulegone were recorded without cells being used at the same time.

It follows from the chromatograms shown in Appendix 3, inter alia, that no detectable p-mentha-8-thiomethyl-3-one is formed (compare 3b with 3e). The pulegone peak in Figures 3d and 3e (retention time 2.7 min.) may be explained by the fact that some of the S-cysteinyl-pulegone dissolves in the extraction agent and is decomposed in the gas chromatograph (160 °C).

The chromatogram of chemically synthesized p-mentha-8-thiol-3-one (Fig. 3a) reveals an isomer ratio of approximately 2:1. The biologically prepared p-mentha-8-thiol-3-one (Fig. 3e) has a completely different ratio of the two isomers which is approximately 9:1.

Example III

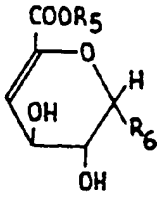
A commercial cocoa mix was used to prepare two different batches of beverage. The first batch is evaluated without any further addition while p-mentha-8-thiol-3-one prepared according to Example II was added to the second batch in the ratio of 20 µg of said p-mentha-8-thiol-3-one to each kilo of cocoa beverage. The beverage containing p-mentha-8-thiol-3-one has a fuller and richer flavour comparing to the beverage without p-mentha-8-thiol-3-one.

Claims

1. Method for preparing thiol compounds, characterized in that cysteine is coupled by means of addition to a compound having the formula $(R_1)(R_2)C=C(R_3)\text{-}Co\text{-}R_4$ in which the symbols R_1-R_4 represent a hydrogen or an optionally saturated and/or heterogeneous hydrocarbon group or, together with the carbon atoms to which the symbols are bonded, form one or two, optionally saturated and/or heterogeneous hydrocarbon ring systems and that subsequently the cysteine conjugate obtained is reacted with β-lyase to form the relevant thiol compounds.

2. The method according to Claim 1, characterized in that cysteine is coupled by means of addition to a compound having formula $(R_1)(R_2)C=C(R_3)\text{-}CO\text{-}R_4$ in which R_1 has the meaning stated in Claim 1, R_2 and R_3 represent a hydrogen atom or an alkyl group containing 1-3 carbon atoms and R_4 represents an optionally heterogeneous hydrocarbon group bonded via an -O- bridge and that subsequently the cysteine conjugate obtained is reacted with β-lyase to form the relevant thiol compounds.

3. The method according to Claim 1, characterized in that cysteine is coupled by means of addition to a compound having the formula:



in which the symbol R_5 represents a hydrogen atom, an alkyl group containing 1-24 carbon atoms or an alkaline ion and R_6 represents a group consisting of 1-7 monosaccharides selected from the group consisting of glucose, mannose, galactose, arabinose, fucose, xylose, rhamnose, uronic acids as well as the acetates, pyruvates, amines and sulfates derived therefrom and that subsequently the cysteine conjugate obtained is reacted with β-lyase to form the relevant thiol compounds.

4. Method according to one or more of the Claims 1-3, characterized in that the cysteine conjugate is split by means of bacterial β-lyase.

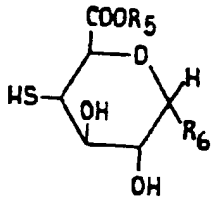
5. Method according to Claim 4, characterized in that β-lyase from Eubacterium limosum is used.

6. Method according to Claims 1, 4, or 5, characterized in that β-lyase is used in the form of bacterial cells.

7. Method according to one or more of the Claims 1-6, characterized in that the flavouring p-mentha-8-thiol-3 one is prepared starting from pulegone.

8. Method according to one or more of the Claims 1-6, characterized in that the compound 16-mercapto-progesterone is prepared starting from 16-dehydroprogesterone.

9. Flavour composition comprising an effective flavouring amount of one or more flavouring compounds of the formula



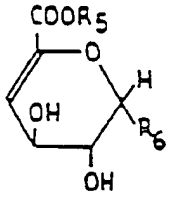
in which the symbol R_5 represents an hydrogen atom, an alkyl group containing 1-24 carbon atoms or an alkaline ion and R_6 represents a group consisting of 1-7 monosaccharides selected from the group consisting of glucose, mannose, galactose, arabinose, fucose, xylose, rhamnose, uronic acids as well as the acetates, pyruvates, amines and sulfates derived therefrom, prepared according to Claim 3, together with customary ingredients.

Patentansprüche

1. Verfahren zur Herstellung Von Thiolverbindungen, dadurch gekennzeichnet, daß Cystein mittels Addition an eine Verbindung der Formel $(R_1)(R_2)C = C(R_3)\text{-}CO\text{-}R_4$ gekuppelt wird, worin die Symbole R_1 -R_4 Wasserstoff oder eine wahlweise gesättigte und/oder heterogene Kohlenwasserstoffgruppe bedeuten oder zusammengenommen mit den Kohlenstoffatomen, an welche die Symbole gebunden sind, eine oder zwei, wahlweise gesättigte und/oder heterogene Kohlenwasserstoffringsysteme bilden, und daß nachfolgend das so erhaltene Cystein-Konjugat mit β-Lyase umgesetzt wird, um die entsprechenden Thiol-Verbindungen zu bilden.

2. Verfahren gemäß Anspruch 1, dadurch gekennzeichnet, daß Cystein mittels Addition an eine Verbindung der Formel $(R_1)(R_2)C=C(R_3)$-CO-R_4 gekuppelt wird, wobei R_1 die in Anspruch 1 angegebene Bedeutung hat, R_2 und R_3 Wasserstoff oder eine Alkylgruppe mit 1-3 Kohlenstoffatomen darstellen und R_4 eine wahlweise heterogene Kohlenwasserstoffgruppe bedeutet, welche über eine -O-Brücke gebunden ist, und daß nachfolgend das so erhaltene Cystein-Konjugat mit β-Lyase umgesetzt wird, um die entsprechenden Thiol-Verbindungen zu bilden.

3. Verfahren gemäß Anspruch 1, dadurch gekennzeichnet, daß Cystein mittels Addition an eine Verbindung der Formel



gekuppelt wird, worin R_5 Wasserstoff, eine Alkylgruppe, enthaltend 1-24 Kohlenstoffatome, oder ein alkalisches Ion darstellt und R_6 eine Gruppe bedeutet, bestehend aus 1-7 Monosacchariden, ausgewählt aus der Gruppe bestehend aus Glucose, Mannose, Galactose, Arabinose, Fucose, Xylose, Rhamnose, Uronsäuren sowie den davon abgeleiteten Acetaten, Pyruvaten, Aminen und Sulfaten, und daß nachfolgend das so erhaltene Cystein-Konjugat mit β-Lyase umgesetzt wird, um die entsprechenden Thiol-Verbindungen zu bilden.

4. Verfahren gemäß einem oder mehreren der Ansprüche 1-3, dadurch gekennzeichnet, daß das Cystein-Konjugat mittels bakterieller β-Lyase gespalten wird.

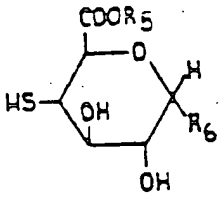
5. Verfahren gemäß Anspruch 4, dadurch gekennzeichnet, daß von Eubacterium limosum stammende β-Lyase verwendet wird.

6. Verfahren gemäß den Ansprüchen 1, 4 oder 5, dadurch gekennzeichnet, daß β-Lyase in Form von Bakterienzellen verwendet wird.

7. Verfahren gemaß einem oder mehreren der Ansprüche 1-6, dadurch gekennzeichnet daß der Geschmacksstoff p-Mentha-8-thiol-3-on, ausgehend von Pulegon, hergestellt wird.

8. Verfahren gemäß einem oder mehreren der Ansprüche 1-6, dadurch gekennzeichnet, daß die Verbindung 16-Mercapto-progesteron, ausgehend von 16-Dehydroprogesteron, hergestellt wird.

9. Geschmackszusammensetzung. umfassend eine geschmacklich wirksame Menge eines oder mehrerer Geschmacksstoffe der Formel



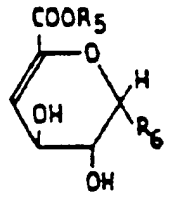
worin R_5 Wasserstoff, eine Alkylgruppe, enthaltend 1-24 Kohlenstoffatome, oder ein alkalisches Ion darstellt und R_6 eine Gruppe bedeutet, bestehend aus 1-7 Monosacchariden, ausgewählt aus der Gruppe bestehend aus Glucose, Mannose, Galactose, Arabinose, Fucose, Xylose, Rhamnose, Uronsäuren sowie den davon abgeleiteten Acetaten, Pyruvaten, Aminen und Sulfaten, hergestellt gemaß Anspruch 3, zusammen mit üblichen Bestandteilen.

Revendicati ns

1. Procédé pour préparer des thiols, caractérisé en ce que la cystéine est couplée au moyen d'une addition à un composé ayant la formule $(R_1)(R_2)C=C(R_3)$-CO-R_4 dans laquelle les symboles R_1 à R_4 représentent un hydrogène ou un groupe hydrocarboné facultativement saturé et/ou hétérogène ou, avec les atomes de carbone auxquels les symboles sont reliés, forment un ou deux systèmes de cycles hydrocarbonés, facultativement saturés et/ou hétérogènes et en ce que l'on fait ensuite réagir le conjugué de la cystéine obtenu avec la β-lyase pour former les thiols correspondants.

2. Procédé selon la revendication 1, caractérisé en ce que la cystéine est couplée au moyen d'une addition à un composé ayant la formule $(R_1)(R_2)C=C(R_3)$-CO-R_4 dans laquelle R_1 a la signification définie dans la revendication 1, R_2 et R_3 représentent un atome d'hydrogène ou un groupe alkyle contenant de 1 à 3 atomes de carbone et R_4 représente un groupe hydrocarboné facultativement hétérogène relié par un pont -O- et en ce que l'on fait ensuite réagir le conjugué de la cystéine obtenu avec de la β-lyase pour former les thiols correspondants.

3. Procédé selon la revendication 1, caractérisé en ce que la cystéine est couplée au moyen d'une addition à un composé ayant la formule:



dans laquelle le symbole R_5 représente un atome d'hydrogène, un groupe alkyle contenant de 1 à 24 atomes de carbone ou un ion alcalin et R_6 represente un groupe composé de 1 à 7 monosaccharides choisis dans le groupe composé du glucose, du mannose, du galactose, de l'arabinose, du fucose, du xylose, du rhamnose, des acides uroniques, aussi bien que des acétates, pyruvates, des amines et des sulfates qui en sont dérivés, et en ce que l'on fait ensuite réagir le conjugué de la cystéine obtenu avec la β-lyase pour former les thiols correspondants.

4. Procédé selon une ou plusieurs des revendications 1 à 3, caractérisé en ce que le conjugué de la cystéine est scindé au moyen de β-lyase bactérienne.

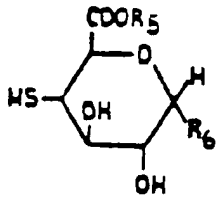
5. Procédé selon la revendication 4, caractérisé en ce que l'on utilise la β-lyase de Eubacterium limosum.

6. Procédé selon les revendications 1, 4, ou 5, caractérisé en ce que la β-lyase est utilisée sous la forme de cellules bactériennes.

7. Procédé selon une ou plusieurs des revendications 1 à 6, caractérisé en ce que la p-mentha-8-thiol-3-one odoriférante est préparée à partir de la pulégone.

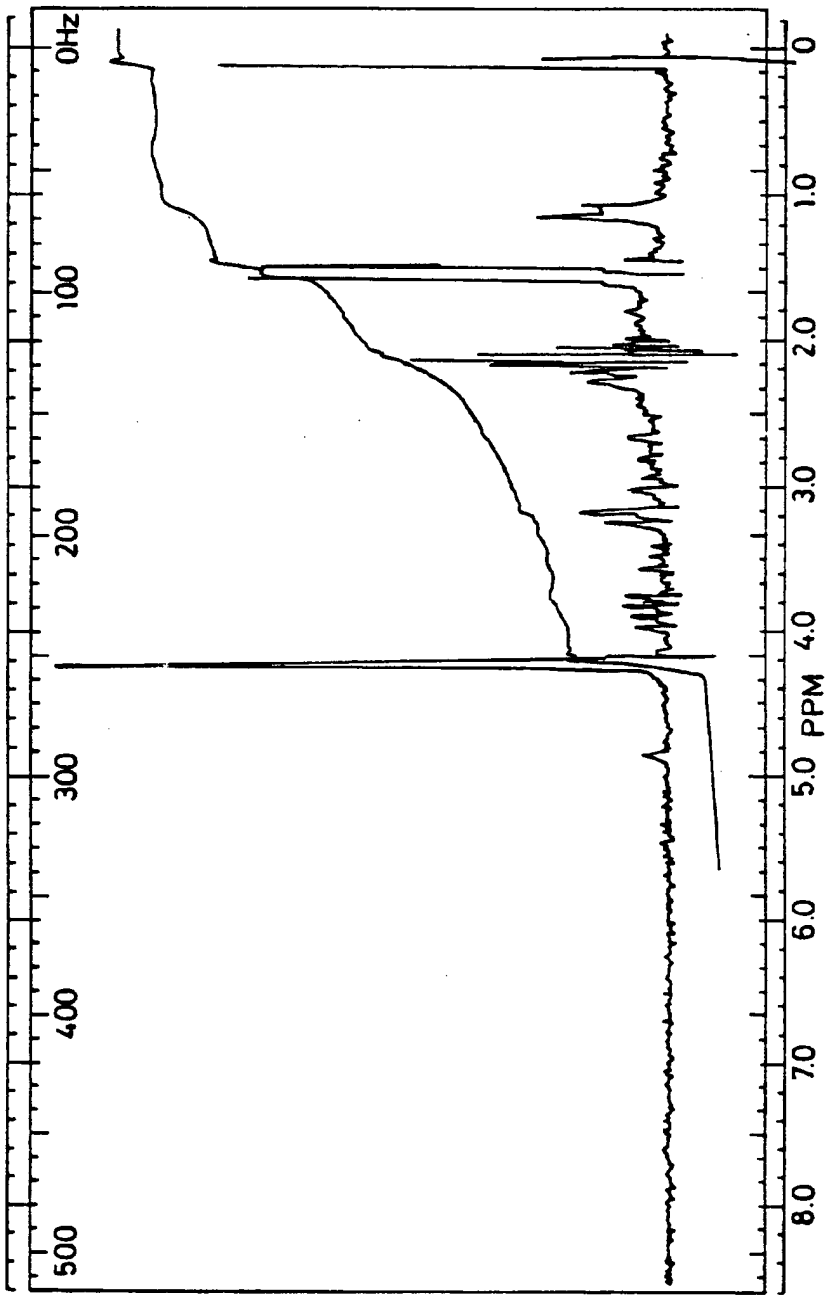
8. Procédé selon une ou plusieurs des revendications 1 à 6, caractérisé en ce que le composé 16-mercapto-progestérone est préparé en partant de la 16-déhydroprogestérone.

9. Composition aromatisante comprenant une quantité effectivement aromatisante d'un ou plusieurs composés aromatisante de formule

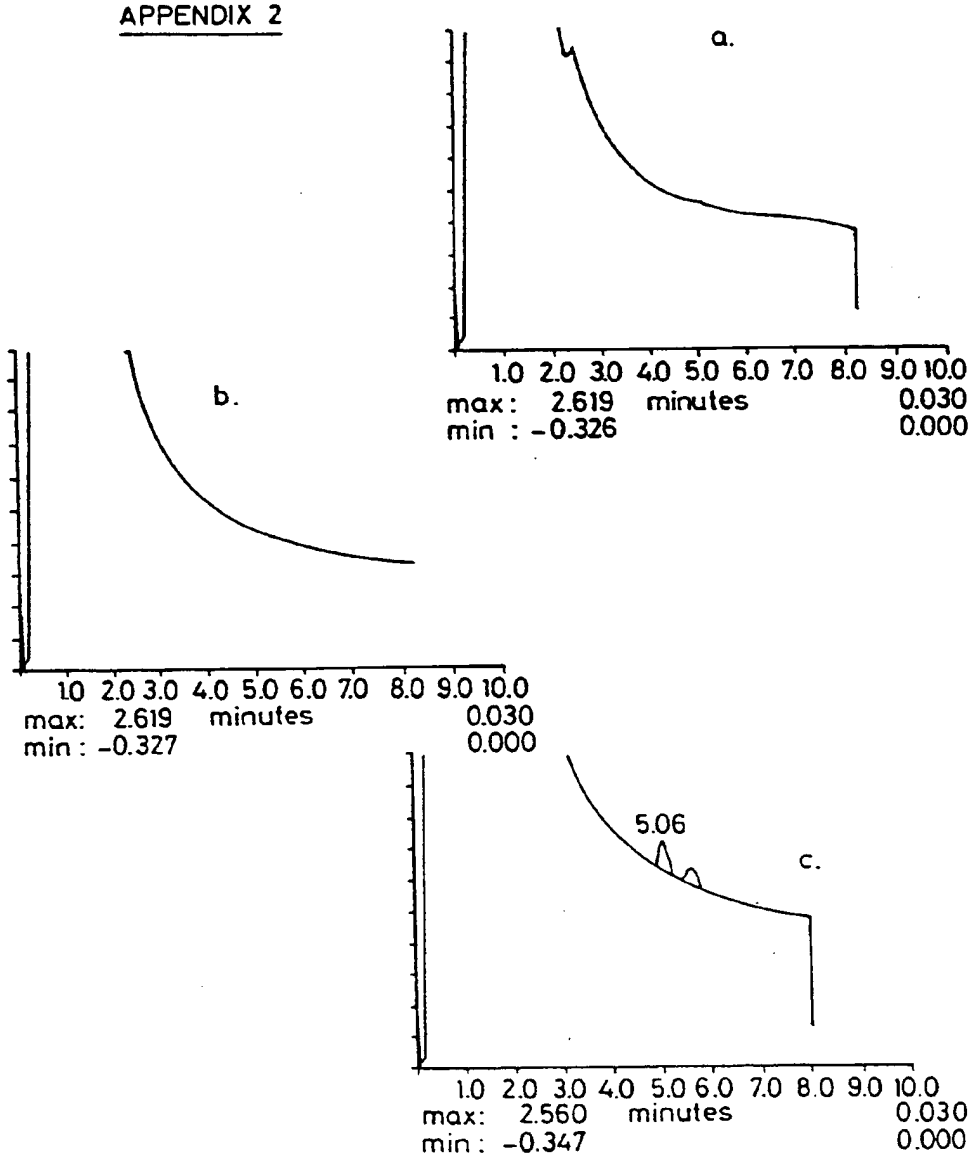


dans laquelle le symbole R_5 représente un atome d'hydrogène, un groupe alkyle contenant de 1 à 24 atomes de carbone ou un ion alcalin et R_6 représente un groupe composé de 1 à 7 monosaccharides choisis dans le groupe composé du glucose, du mannose, du galactose, de l'arabinose, du fucose, du xylose, du rhamnose, des acides uroniques aussi bien que des acétates, des pyruvates, des amines et des sulfates qui en sont dérivés, préparés selon la revendication 3, avec les ingrédients habituels.

APPENDIX 1



APPENDIX 2

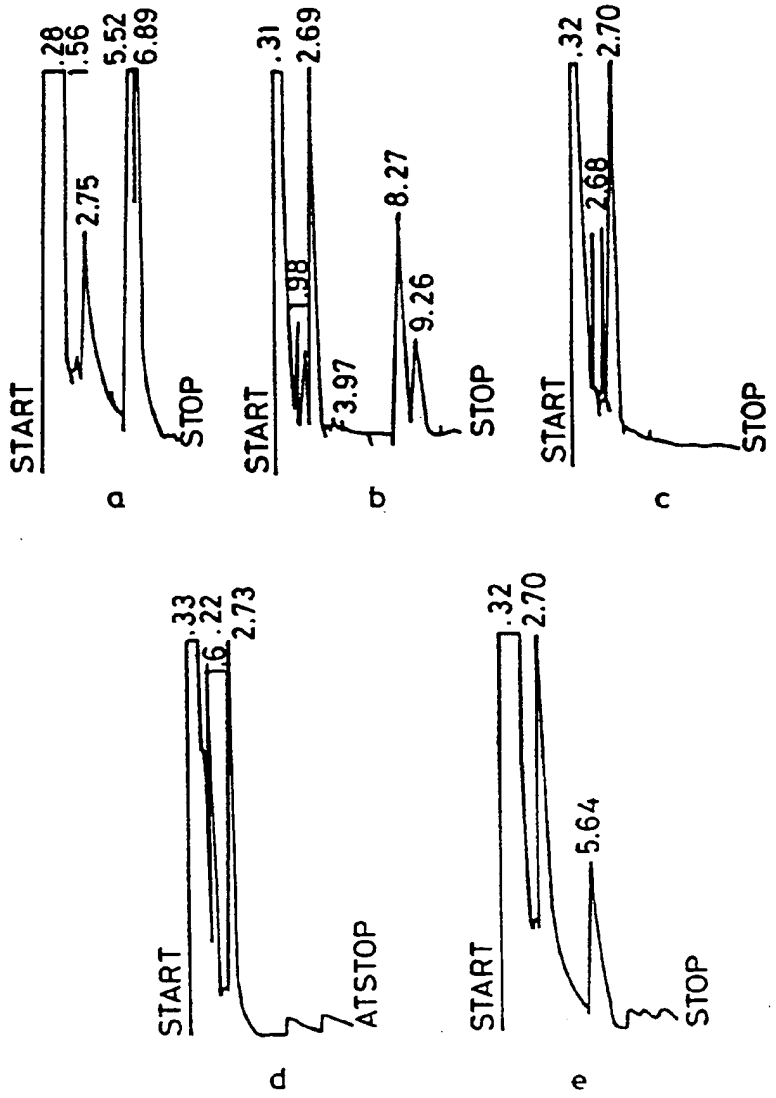


Chromatograms of the samples of Example I

a. sample no. 1
b. sample no. 2
c. sample no. 3

Note: The samples have been extracted with CHCl (1 to 1)
1µl of this extract has been analysed by gas chromatography.

APPENDIX 3



Chromatograms of some extracts of example II

a	0.02% TF in buffer	(recorder : 1 mV F.S.)
b	0.02% TFM in buffer	(recorder : 2mV F.S.)
c	0.02% pulegone in buffer	(recorder : 2mV F.S.)
d	0.03% S-cysteinyl-pulegone in buffer	(recorder : 1/2 mV F.S.)
e	Sample B	(recorder : 1/2 mV F.S.)

Note : The samples have been extracted with CH_2Cl_2 (4 to 1).
0.4 µl of this extract was analysed by gas chromatography.

F.S. = full scale TF= p-mentha-8-thiol-3 one
TFM = p-mentha-8-thiomethyl-3-one